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(71) Applicant *(for all designated States except US)*: MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MARCY, Alice [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). SALOWE, Scott, P. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). WISNIEWSKI, Douglas [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

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(54) Title: A HIGH THROUGHPUT ASSAY USING FUSION PROTEINS

(57) Abstract

This application describes a high throughput assay for screening for compounds capable of binding to a fusion protein which consists of a target protein and an FK506-binding protein. The method for preparing the DNA encoding for the fusion protein and for expressing that DNA is also described in the application. The invention also discloses the recombinant DNA and protein sequences for several fusion proteins.

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TITLE OF THE INVENTION

A HIGH THROUGHPUT ASSAY USING FUSION PROTEINS

BACKGROUND OF THE INVENTION

Src homology 2 (SH2) domains are a family of homologous protein domains that share the common property of recognizing phosphorylated tyrosine residues in specific peptide contexts. They have routinely been expressed in *E. coli* as fusion proteins with glutathione-S-transferase (GST). This usually provides high level expression and straightforward affinity purification on glutathione-Sepharose. Ligand binding is then assayed by incubating the GST/SH2 with a radiolabeled phosphopeptide, precipitating the complex with glutathione-Sepharose, washing the beads, and then counting the beads to determine bound radioactivity [Isakov et al., *J. Exp. Med.*, 181, 375-380 (1995); Piccione et al., *Biochemistry*, 32, 3197-3202 (1993); Huyer et al., *Biochemistry*, 34, 1040-1049 (1995)]. There are several disadvantages to this procedure, particularly when applied to high-throughput screening for agonists, antagonists, or inhibitors as new leads for drug development. First, the radiolabeling of the peptide is carried out either enzymatically with a kinase and [^{32}P]ATP or chemically with [^{125}I]Bolton-Hunter reagent. In both cases, the isotopes are short-lived and thus require frequent preparation of material. In the case of enzymatic phosphorylation, the appropriate kinase must also be available in sufficient quantity to generate enough material for screening purposes. Second, the protocol requires separation of bound complex from free phosphopeptide by washing of the glutathione-Sepharose beads. This is a nonequilibrium procedure that risks dissociation of the bound ligand, particularly when off-rates are fast. Thus, there is the possibility of misleading results. Finally, due to the number of manipulations and centrifugations involved, the protocol is very tedious to conduct manually and is not readily adaptable to robotic automation to increase throughput.

Two additional methods for measuring the interaction of proteins and ligands that have been applied to SH2 domains are

biospecific interaction analysis using surface plasmon resonance and isothermal titration calorimetry (Felder et al., *Mol. Cell. Biol.*, 13, 1449-1455 (1993); Panayotou et al., *Mol. Cell. Biol.*, 13, 3567-3576 (1993); Payne et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90, 4902-4906 (1993); Morelock et al., *J. Med. Chem.* 38, 1309-18 (1995); Ladbury et al., *Proc. Natl. Acad. Sci. U.S.A.*, 92, 3199-3203 (1995); Lemmon et al., *Biochemistry*, 33, 5070-5076 (1994)). These techniques do not require a particular fusion partner for the SH2 domain, but do require sophisticated instrumentation that is not amenable to high throughput screening.

SUMMARY OF THE INVENTION

The instant invention covers a method of screening for compounds capable of binding to a fusion protein which comprises combining a test compound, a tagged ligand, a fusion protein (target protein, peptide linker and FK506-binding protein), and a radiolabeled ligand in a coated microscintillation plate, and then measuring the scintillation counts attributable to the binding of the tagged ligand to the fusion protein in the presence of the test compound relative to a control assay in the absence of the test compound, so as to determine the effect the test compound has on the binding of the tagged ligand. Also within the scope of this invention are the processes for preparing and expressing the recombinant DNA encoding a fusion protein. This invention further relates to the recombinant DNA expression vector capable of expressing the fusion protein. This invention further relates to a process for purifying the recombinant fusion protein. This invention provides an immediate means of making use of microscintillation plate technology for the functional assay of ligand binding to a single or multiple signal transduction domain(s), for example a phosphopeptide binding to an SH2 domain. The present invention does not require specialized radiochemical synthesis and is readily adaptable to robotic automation for high capacity screening for agonists, antagonists, and/or inhibitors.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1.

A.) Binding of the streptavidin microscintillation plate, biotinylated ligand and the fusion protein (SH2:FKBP), which emits a detectable signal; and

B.) Binding of the test compound and the fusion protein (SH2:FKBP), which results in no signal detection .

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of screening for compounds which preferentially bind to a target protein.

An embodiment of this invention is a method of screening for compounds capable of binding to a fusion protein which comprises the steps of:

a) mixing a test compound, a tagged ligand, the fusion protein, and a radiolabeled ligand;
b) adding the mixture to a coated microscintillation plate;
c) incubating the mixture for between about 1 hour and about 24 hours;
d) measuring the plate-bound counts attributable to the binding of the tagged ligand to the fusion protein in the presence of the test compound using scintillation counting; and
e) determining the binding of the tagged ligand to the fusion protein in the presence of the test compound relative to a control assay run in the absence of the test compound.

A second embodiment of this invention is a process for preparing a recombinant DNA expression vector encoding for a fusion protein comprising the steps of:

a) removing the stop codon on DNA encoding for an FK506-binding protein;
b) synthesizing a modified DNA fragment on the DNA encoding for the FK506-binding protein which encodes for a peptide linker;

c) digesting an expression vector at cloning sites;

d) cloning the modified DNA fragment encoding for the FK506-binding protein with a peptide linker into the digested expression vector to generate a recombinant DNA expression vector encoding for FK506-binding protein with a peptide linker; and

e) cloning DNA encoding for a target protein into a recombinant DNA expression vector encoding for FK506-binding protein with a peptide linker to produce the recombinant DNA expression vector encoding for the fusion protein.

A third embodiment of this invention is a process for expressing recombinant DNA encoding for a fusion protein in an expression vector comprising the steps of:

a) transforming a host cell with the fusion protein expression vector;

b) inducing expression of the fusion protein in the host cell;

c) recovering the fusion protein from the host cell; and

d) purifying the fusion protein.

A fourth embodiment of this invention is a process for purifying an isolated FKBP-SH2 fusion protein, comprising the steps of:

a) preparing an affinity matrix consisting of biotinylated phosphopeptide coupled to avidin or streptavidin immobilized on a solid support;

b) preparing a freeze/thaw extract from cells expressing the fusion protein;

c) loading the extract onto the affinity matrix and washing off unbound protein; and

d) eluting the desired fusion protein with phenyl phosphate.

The term "fusion protein" refers to a "target protein" fused to an "FK506-binding protein" (FKBP), the two proteins being separated by a "peptide linker".

A "peptide linker" may consist of a sequence containing from about 1 to about 20 amino acids, which may or may not include the sequence for a protease cleavage site. An example of a peptide linker which is a protease cleavage site is represented by the amino acid sequence GLPRGS.

The term "target protein" refers to any protein that has a defined ligand. Included within this definition of target protein are single and multiple signal transduction domains, such as, but not limited to, Src homology 1 (SH1), Src homology 2 (SH2), Src homology 3 (SH3), and pleckstrin homology (PH) domains [Hanks & Hunter, *FASEB J.*, 9, 576-596 (1995); Bolen, *Curr. Opin. Immunol.*, 7, 306-311 (1995); Kuriyan & Cowburn, *Curr. Opin. Struct. Biol.*, 3, 828-837 (1993); Cohen et al., *Cell*, 80, 237-248 (1995)]. The term "SH1 domain" refers to a family of homologous protein domains that bind ATP and catalyze tyrosine phosphorylation of peptide and protein substrates. The term "SH2 domain" refers to a family of homologous protein domains that share the common property of recognizing phosphorylated tyrosine residues in specific peptide contexts. The term "SH3 domain" refers to a family of homologous protein domains that share the common property of recognizing polyproline type II helices. The term "PH domain" refers to a family of homologous protein domains that mediate both protein-protein and protein-lipid interactions. Examples of SH2 domains which may be utilized in the method of the invention include, but are not limited to, the single and tandem SH2 domains present in the tyrosine kinases ZAP, SYK and LCK. The DNA sequences were obtained from GenBank, National Center for Biotechnology Information, National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894. The Accession Numbers for the sequences are: human ZAP (L05148); human SYK (L28824) and human LCK (X13529).

The term "tagged ligand" refers to a biotinylated or epitope tagged ligand for the target protein.

The term "radiolabeled ligand" refers to a [^{3}H]-, [^{125}I]-, [^{14}C]-, [^{35}S]-, [^{32}P]-, or [^{33}P]-labeled ligand which binds to the FKBP.

An example of a radiolabeled ligand useful in the instant invention is [^{3}H]-dihydroFK506.

The term "coated microscintillation plates" refers to streptavidin-coated microscintillation plates when the tagged ligand is biotinylated, and to anti-epitope antibody bound to anti-antibody-coated or protein A-coated microscintillation plates when the tagged ligand is epitope-tagged. Examples of coated microscintillation plates useful in the instant invention are streptavidin-coated, sheep anti-rabbit-coated, and goat anti-mouse-coated FlashPlate Plus (DuPont-NEN). Additional coatings, including but not limited to protein A, may be applied to uncoated FlashPlates by methods known to those skilled in the art.

The term "control assay" refers to the assay when performed in the presence of the tagged ligand, the fusion protein, the radiolabeled ligand and the coated microscintillation plates, but in the absence of the test compound.

The term FK506-binding proteins may include, but are not limited to, the below listed FKBPs and FKBP homologues, which include a citation to the references which disclose them. This list is not intended to limit the scope of the invention.

Mammalian

FKBP-12	Galat et al., *Eur. J. Biochem.*, 216:689-707 (1993).
FKBP-12.6	Wiederrecht, G. and F. Etzkorn *Perspectives in Drug Discovery and Design* , 2:57-84 (1994).
FKBP-13	Galat et al., *supra*; Wiederrecht and Etzkorn, *supra*.
FKBP-25	Galat et al., *supra*; Wiederrecht and Etzkorn, *supra*.
FKBP-38	Wiederrecht and Etzkorn, *supra*.
FKBP-51	Baughman et al., *Mol. Cell. Biol.*, 8, 4395-4402(1995) .
FKBP-52	Galat et al., *supra*.

Bacteria

Legionella pneumophilia	Galat et al., *supra.*
Legionella micadei	Galat et al., *supra.*
Chlamydia trachomatis	Galat et al., *supra.*
E. coli fkpa	Horne, S.M. and K.D. Young, *Arch. Microbiol.*, 163:357-365 (1995).
E. coli slyD	Roof et al., *J. Biol. Chem.* 269:2902-2910 (1994).
E. coli orf149	Trandinh et al., *FASEB J.* 6:3410-3420 (1992).
Neisseria meningitidis	Hacker, J. and G. Fischer, *Mol. Micro.*, 10:445-456 (1993).
Streptomyces chrysomallus	Hacker and Fischer, *supra.*

Fungal

yeast FKBP-12	Cardenas et al., *Perspectives in Drug Discovery and Design* , 2:103-126 (1994).
yeast FKBP-13	Cardenas et al., *supra.*
yeast NPR1(FPR3)	Cardenas et al., *supra.*
Neurospora	Galat et al., *supra.*

A variety of host cells may be used in this invention, which include, but are not limited to, bacteria, yeast, bluegreen algae, plant cells, insect cells and animal cells.

Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express genes in a variety of host cells, such as, bacteria, yeast, bluegreen algae, plant cells, insect cells and animal cells.

Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An

appropriately constructed expression vector may contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available vectors suitable for FKBP fusion protein expression include, but are not limited to pBR322 (Promega), pGEX (Amersham), pT7 (USB), pET (Novagen), pIBI (IBI), pProEX-1 (Gibco/BRL), pBluescript II (Stratagene), pTZ18R and pTZ19R (USB), pSE420 (Invitrogen), pVL1392 (Invitrogen), pBlueBac (Invitrogen), pBAcPAK (Clontech), pHIL (Invitrogen), pYES2 (Invitrogen), pCDNA (Invitrogen), pREP (Invitrogen) or the like.

The expression vector may be introduced into host cells via any one of a number of techinques including but not limited to transformation, transfection, infection, protoplast fusion, and electroporation.

E. coli containing an expression plasmid with the target gene fused to FKBP are grown and appropriately induced. The cells are then pelleted and resuspended in a suitable buffer. Although FKBP-12 lacks sequences that specifically direct it to the periplasm, FKBP fusions are primarily located there and can be released by a standard freeze/thaw treatment of the cell pellet. Following centrifugation, the resulting supernatant contains >80% pure FKBP fusion, which if desired can be purified further by conventional methods. Alternatively, the assay is not dependent on pure protein and the initial periplasmic preparation may be used directly. A thrombin site located between FKBP and the target protein can be used as a means to cleave FKBP from the fusion; such cleaved material may be a suitable negative control for subsequent assays.

A fusion protein which contains a single or multiple SH2 domain(s) may be purified by preparing an affinity matrix consisting of biotinylated phosphopeptide coupled to avidin or streptavidin immobilized on a solid support. A freeze/thaw extract is prepared from the cells which express the fusion protein and is loaded onto the affinity matrix. The desired fusion protein is then specifically eluted with phenyl phosphate.

To assay the formation of a complex between a target protein and its ligand, the tagged ligand is mixed with the FKBP fusion protein in a suitable buffer in the presence of the radiolabeled ligand. After a suitable incubation period to allow complex formation to occur, the mixture is transferred to a coated microscintillation plate to capture the tagged ligand and any bound fusion protein. The plate is sealed, incubated for a sufficient period to allow the capture to go to completion, then counted in a multiwell scintillation counter. Screening for agonists/antagonists/inhibitors is carried out by performing the initial incubation prior to the capture step in the microscintillation plate in the presence of a test compound(s) to determine whether they have an effect upon the binding of the tagged ligand to the fusion protein. This principle is illustrated in Figure 1.

The present invention can be understood further by the following examples, which do not constitute a limitation of the invention.

EXAMPLE 1

Process for Preparing the FKBP fusion cloning vector

General techniques for modifying and expressing genes in various host cells can be found in Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. Current Protocols in Molecular Biology (John Wiley & Sons, New York, New York, 1989). Sequences for a 3'- altered FKBP fragment that contained a glycine codon (GGT) in place of the stop (TGA) codon followed by a sequence encoding a thrombin site (Leu-Val-Pro-Arg) and *Bam*HI restriction site (GAATTC) were amplified using the polymerase chain

reaction (PCR). The PCR reaction contained the following primers:5'-GATCGCCATGGGAGTGCAGGTGGAAACCATCTCCCCA-3' and 5'-TACGAATTCTGGCGTGGATCCACGCGGAACCAGACCTTCCAGTTTTAG-3' and a plasmid containing human FKBP-12 as the template. The resulting 367 base pair amplification product was ligated into the vector pCRII (Invitrogen) and the ligation mixture transformed into competent *Escherichia coli* cells. Clones containing an insert were identified using PCR with flanking vector primers. Dideoxy DNA sequencing confirmed the nucleotide sequence of one positive isolate. The altered 338 base pair FKBP fragment was excised from the pCRII plasmid using *Nco*I and *BamH*I and ligated into *Nco*I and*Bam*HI digested pET9d (Novagen) plasmid. Competent *E. coli* were transformed with the ligation mixture, and colonies containing the insert were identified using PCR with primers encoding for flanking vector sequences. The FKBP fusion cloning vector is called pET9dFKBPt.

EXAMPLE 2

Process for Preparing the FK-ZAP fusion expression vector

A DNA fragment encoding for the tandem SH2 domains of ZAP-70 was prepared by PCR to contain a *Bam*HI site at the 5'-end such that the reading frame was conserved with that of FKBP in the fusion vector. At the 3'-end, the fragment also incorporated a stop codon followed by a *Bam*HI site. The PCR reaction contained Molt-4 cDNA (Clontech) and the following primers: 5'-ATTAGGATCCATGCCAGATCCTGCAGCTCACCTGCCCT-3' and 5'-ATATGGATCCTTACCAGAGGCGTTGCT-3'. The fragment was cloned into a suitable vector, sequenced, digested with *Bam*HI, and the insert containing the SH2 domains ligated to *Bam*HI treated pET9dFKBPt, and transformed into *E. coli*. Clones containing inserts in the correct orientation were identified by PCR or restriction enzyme analysis. Plasmid DNA was prepared and used to transform BL21(DE3) cells.

EXAMPLE 3

Process for Preparing the FK-SYK fusion expression vector

The expression vector for the tandem SH2 domains of Syk fused to FKBP was prepared as in Example 2 except that the PCR reaction contained Raji cell cDNA (Clontech) and the following primers: 5'-CAATAGGATCCATGGCCAGCAGCGGCATGGCTGA-3' and 5'-GACCTAGGATCCCTAATTAACATTTCCCTGTGTGCCGAT-3'.

EXAMPLE 4

Process for Preparing the FK-LCK fusion expression vector

The expression vector for the SH2 domain of Lck fused to FKBP was prepared as in Example 2 except that the PCR reaction contained Molt-4 cDNA (Clontech) and the following primers:
5'-ATATGGATCCATGGCGAACAGCCTGGAGCCCGAACCCT-3'
and
5'-ATTAGGATCCTTAGGTCTGGCAGGGGCGGCTCAACCGTG TGCA-3'.

EXAMPLE 5

FK-ZAP

Step A: Process for Expression of FK-ZAP

E. coli BL21(DE3) cells containing the pET9dFKBPt/ ZapSH2 plasmid were grown in Luria-Bertani (LB) media containing 50 microgram/ml kanamycin at about 37 degrees C until the optical density measured at 600 nm was about 0.5-1.0. Expression of the FK-ZAP fusion protein was induced with 0.1 mM isopropyl beta-thiogalactopyranoside and the cells were grown for another 3-5 hr at about 30 degrees C. They were pelleted at 4400 x g for about 10 min at about 4 degrees C and resuspended in 2% of the original culture volume with 100 mM tris pH 8.0 containing 1 microgram/ml each aprotinin,

pepstatin, leupeptin, and bestatin. The resuspended pellet was frozen at about -20 degrees C until further purification.

Step B: Process for Purification of FK-ZAP

The affinity matrix for purification of FK-ZAP was prepared by combining agarose-immobilized avidin with excess biotinylated phosphopeptide derived from the ζ1 ITAM sequence of the human T-cell receptor, biotinyl-GSNQLpYNELNLGRREEpYDVLDK, and washing out unbound peptide. Frozen cells containing FK-ZAP were thawed in warm water, refrozen on dry ice for about 25 min., then thawed again. After the addition of 0.1% octyl glucoside, 1 mM dithiothreitol (DTT) and 500 mM NaCl, the extract was centrifuged at 35,000 x g for approximately 30 minutes. The supernatant was loaded onto the phosphopeptide affinity column, at about 4° and washed with phosphate buffered saline containing 1 mM DTT and 0.1% octyl glucoside. FK-ZAP was eluted with 200 mM phenyl phosphate in the same buffer at about 37°. The protein pool was concentrated and the phenyl phosphate removed on a desalting column. The purified FK-ZAP was stored at about -30° in 10 mM HEPES/150 mM NaCl/1 mM DTT/0.1 mM EDTA/10% glycerol.

EXAMPLE 6

FK-SYK

E. coli BL21(DE3) cells containing the pET9dFKBPt/SykSH2 plasmid were grown, induced, and harvested as described in Example 5. FK-SYK was purified using the same affinity matrix and methodology described in Example 5.

EXAMPLE 7

FK-LCK

E. coli BL21(DE3) cells containing the pET9dFKBPt/LckSH2 plasmid were grown, induced, and harvested as described in

Example 5. The affinity matrix for purification of FK-LCK was prepared by combining agarose-immobilized avidin with excess biotinyl- EPQpYEEIPIYL, and washing out unbound peptide. The remaining methodology for purification was the same as Example 5.

EXAMPLE 8

Assay of phosphopeptide binding to FK-ZAP

Assays were conducted at ambient temperature in a buffer consisting of 25 mM HEPES, 10 mM DTT, 0.01% TWEEN-20, pH 7.0. 300 μl of a mixture of buffer and varying amounts of biotinyl-phosphopeptide were combined with 25 μl of FK-ZAP protein and 50 μl of [^{3}H]-dihydroFK506 (DuPont NEN) in microfuge tubes. A 150 μl portion of each assay was then transferred to the well of a streptavidin-coated FlashPlate Plus (DuPont-NEN) and an additional 50 μl of buffer was added. Final concentrations of the assay components were:

0-50 nM biotinyl-GSNQLpYNELNLGRREEpYDVLDK
100 nM FK-ZAP fusion protein
25 nM [^{3}H]-dihydroFK506

The plate was sealed and incubated 20 hours. Plate-bound radioactivity was measured at various timepoints in a Packard Topcount microplate scintillation counter.

EXAMPLE 9

Method of Screening for Antagonists of FK-ZAP

Assays are conducted at ambient temperature in a buffer consisting of 25 mM HEPES, 10 mM DTT, 0.01% TWEEN-20, pH 7.0. 10 μl of a DMSO solution of test compound(s) and 120 μl of biotinyl-phosphopeptide stock solution are dispensed into the wells of a standard 96-well plate. Next, 20 μl of a mixture of FK-ZAP protein and [^{3}H]-dihydroFK506 (DuPont NEN) are added to each test well. The assays are then transferred to the wells of a streptavidin-coated FlashPlate (DuPont NEN). Final concentrations of the assay components are:

25 nM biotinyl-GSNQLpYNELNLGRREEpYDVLDK
25 nM FK-ZAP fusion protein
10 nM [^{3}H]-dihydroFK506
5% DMSO

The plate is sealed and incubated between 1 and 8 hours. Bead-bound radioactivity is then measured in a Packard Topcount microplate scintillation counter.

EXAMPLE 10

Method of Screening for Antagonists of FK-SYK

The assays are conducted as set forth in Example 9, except that FK-SYK replaces FK-ZAP.

EXAMPLE 11

Method of Screening for Antagonists of FK-LCK

The assays are conducted as set forth in Example 9, except that FK-LCK replaces FK-ZAP and the tagged ligand is 25 nM biotinyl-EPQpYEEIPIYL.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Salowe, Scott P.
Marcy, Alice I.
Wisniewski, Douglas

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(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Valerie J. Camara
(B) STREET: 126 E. Lincoln Avenue, P.O. Box 2000
(C) CITY: Rahway
(D) STATE: NJ
(E) COUNTRY: U.S.A.
(F) ZIP: 07065

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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Camara, Valerie J.
(B) REGISTRATION NUMBER: 35,090
(C) REFERENCE/DOCKET NUMBER: 19524

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (908) 594-3902
(B) TELEFAX: (908) 594-4720

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1137 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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GAAGAAGGGG TTGCCCAGAT GAGTGTGGGT CAGAGAGCCA AACTGACTAT ATCTCCAGAT 240
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AAGTTCCTGC TGAGGCCGCG GAAGGAGCAG GGCACATACG CCCTGTCCCT CATCTATGGG 960
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GGCACCAAGT TTGACACGCT CTGGCAGCTG GTGGAGTATC TGAAGCTGAA GGCGGACGGG 1080
CTCATCTACT GCCTGAAGGA GGCCTGCCCC AACAGCAGTG CCAGCAACGC CTCTTAA 1137

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGGAGTGC AGGTGGAAAC CATCTCCCCA GGAGATGGAC GCACCTTCCC CAAGCGCGGC 60

CAGACCTGCG TGGTGCACTA CACCGGGATG CTTGAAGATG GAAAGAAATT TGATTCCTCC 120

CGGGACAGAA ACAAGCCCTT TAAGTTTATG CTAGGCAAGC AGGAGGTGAT CCGAGGCTGG 180

GAAGAAGGGG TTGCCCAGAT GAGTGTGGGT CAGAGAGCCA AACTGACTAT ATCTCCAGAT 240

TATGCCTATG GTGCCACTGG GCACCCAGGC ATCATCCCAC CACATGCCAC TCTCGTCTTC 300

GATGTGGAGC TTCTAAAACT GGAAGGTCTG GTTCCGCGTG GATCCATGGC CAGCAGCGGC 360

ATGGCTGACA GCGCCAACCA CCTGCCCTTC TTTTTCGGCA ACATCACCCG GGAGGAGGCA 420

GAAGATTACC TGGTCCAGGG GGGCATGAGT GATGGGCTTT ATTTGCTGCG CCAGAGCCGC 480

AACTACCTGG GTGGCTTCGC CCTGTCCGTG GCCCACGGGA GGAAGGCACA CCACTACACC 540

ATCGAGCGGG AGCTGAATGG CACCTACGCC ATCGCCGGTG GCAGGACCCA TGCCAGCCCC 600

GCCGACCTCT GCCACTACCA CTCCCAGGAG TCTGATGGCC TGGTCTGCCT CCTCAAGAAG 660

CCCTTCAACC GGCCCCAAGG GGTGCAGCCC AAGACTGGGC CCTTTGAGGA TTTGAAGGAA 720

AACCTCATCA GGGAATATGT GAAGCAGACA TGGAACCTGC AGGGTCAGGC TCTGGAGCAG 780

GCCATCATCA GTCAGAAGCC TCAGCTGGAG AAGCTGATCG CTACCACAGC CCATGAAAAA 840

ATGCCTTGGT TCCATGGAAA AATCTCTCGG GAAGAATCTG AGCAAATTGT CCTGATAGGA 900

TCAAAGACAA ATGGAAAGTT CCTGATCCGA GCCAGAGACA ACAACGGCTC CTACGCCCTG 960

TGCCTGCTGC ACGAAGGGAA GGTGCTGCAC TATCGCATCG ACAAAGACAA GACAGGGAAG 1020

CTCTCCATCC CCGAGGGAAA GAAGTTCGAC ACGCTCTGGC AGCTAGTCGA GCATTATTCT 1080

TATAAAGCAG ATGGTTTGTT AAGAGTTCTT ACTGTCCCAT GTCAAAAAAT CGGCACACAG 1140

GGAAATGTTA ATTAG 1155

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGGAGTGC AGGTGGAAAC CATCTCCCCA GGAGATGGAC GCACCTTCCC CAAGCGCGGC 60

```
CAGACCTGCG TGGTGCACTA CACCGGGATG CTTGAAGATG GAAAGAAATT TGATTCCTCC    120
CGGGACAGAA ACAAGCCCTT TAAGTTTATG CTAGGCAAGC AGGAGGTGAT CCGAGGCTGG    180
GAAGAAGGGG TTGCCCAGAT GAGTGTGGGT CAGAGAGCCA AACTGACTAT ATCTCCAGAT    240
TATGCCTATG GTGCCACTGG GCACCCAGGC ATCATCCCAC CACATGCCAC TCTCGTCTTC    300
GATGTGGAGC TTCTAAAACT GGAAGGTCTG GTTCCGCGTG GATCCATGGC GAACAGCCTG    360
GAGCCCGAAC CCTGGTTCTT CAAGAACCTG AGCCGCAAGG ACGCGGAGCG GCAGCTCCTG    420
GCGCCCGGGA ACACTCACGG CTCCTTCCTC ATCCGGGAGA GCGAGAGCAC CGCGGGATCG    480
TTTTCACTGT CGGTCCGGGA CTTCGACCAG AACCAGGGAG AGGTGGTGAA ACATTACAAG    540
ATCCGTAATC TGGACAACGG TGGCTTCTAC ATCTCCCCTC GAATCACTTT TCCCGGCCTG    600
CATGAACTGG TCCGCCATTA CACCAATGCT TCAGATGGGC TGTGCACACG GTTGAGCCGC    660
CCCTGCCAGA CCTAA                                                     675
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 378 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg Thr Phe
1               5                   10                  15

Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met Leu Glu
            20                  25                  30

Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg Asn Lys Pro Phe Lys
        35                  40                  45

Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu Gly Val
    50                  55                  60

Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser Pro Asp
65                  70                  75                  80

Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro His Ala
                85                  90                  95
```

```
Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu Gly Leu Val Pro
            100                 105                 110

Arg Gly Ser Met Pro Asp Pro Ala Ala His Leu Pro Phe Phe Tyr Gly
        115                 120                 125

Ser Ile Ser Arg Ala Glu Ala Glu Glu His Leu Lys Leu Ala Gly Met
    130                 135                 140

Ala Asp Gly Leu Phe Leu Leu Arg Gln Cys Leu Arg Ser Leu Gly Gly
145                 150                 155                 160

Tyr Val Leu Ser Leu Val His Asp Val Arg Phe His His Phe Pro Ile
                165                 170                 175

Glu Arg Gln Leu Asn Gly Thr Tyr Ala Ile Ala Gly Gly Lys Ala His
            180                 185                 190

Cys Gly Pro Ala Glu Leu Cys Glu Phe Tyr Ser Arg Asp Pro Asp Gly
        195                 200                 205

Leu Pro Cys Asn Leu Arg Lys Pro Cys Asn Arg Pro Ser Gly Leu Glu
    210                 215                 220

Pro Gln Pro Gly Val Phe Asp Cys Leu Arg Asp Ala Met Val Arg Asp
225                 230                 235                 240

Tyr Val Arg Gln Thr Trp Lys Leu Glu Gly Glu Ala Leu Glu Gln Ala
                245                 250                 255

Ile Ile Ser Gln Ala Pro Gln Val Glu Lys Leu Ile Ala Thr Thr Ala
            260                 265                 270

His Glu Arg Met Pro Trp Tyr His Ser Ser Leu Thr Arg Glu Glu Ala
        275                 280                 285

Glu Arg Lys Leu Tyr Ser Gly Ala Gln Thr Asp Gly Lys Phe Leu Leu
    290                 295                 300

Arg Pro Arg Lys Glu Gln Gly Thr Tyr Ala Leu Ser Leu Ile Tyr Gly
305                 310                 315                 320

Lys Thr Val Tyr His Tyr Leu Ile Ser Gln Asp Lys Ala Gly Lys Tyr
                325                 330                 335

Cys Ile Pro Glu Gly Thr Lys Phe Asp Thr Leu Trp Gln Leu Val Glu
            340                 345                 350

Tyr Leu Lys Leu Lys Ala Asp Gly Leu Ile Tyr Cys Leu Lys Glu Ala
        355                 360                 365

Cys Pro Asn Ser Ser Ala Ser Asn Ala Ser
    370                 375
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 384 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg Thr Phe
1               5                   10                  15

Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met Leu Glu
            20                  25                  30

Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg Asn Lys Pro Phe Lys
        35                  40                  45

Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu Gly Val
    50                  55                  60

Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser Pro Asp
65                  70                  75                  80

Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro His Ala
                85                  90                  95

Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu Gly Leu Val Pro
            100                 105                 110

Arg Gly Ser Met Ala Ser Ser Gly Met Ala Asp Ser Ala Asn His Leu
        115                 120                 125

Pro Phe Phe Phe Gly Asn Ile Thr Arg Glu Glu Ala Glu Asp Tyr Leu
    130                 135                 140

Val Gln Gly Gly Met Ser Asp Gly Leu Tyr Leu Leu Arg Gln Ser Arg
145                 150                 155                 160

Asn Tyr Leu Gly Gly Phe Ala Leu Ser Val Ala His Gly Arg Lys Ala
                165                 170                 175

His His Tyr Thr Ile Glu Arg Glu Leu Asn Gly Thr Tyr Ala Ile Ala
            180                 185                 190

Gly Gly Arg Thr His Ala Ser Pro Ala Asp Leu Cys His Tyr His Ser
        195                 200                 205

Gln Glu Ser Asp Gly Leu Val Cys Leu Leu Lys Lys Pro Phe Asn Arg
    210                 215                 220
```

```
Pro Gln Gly Val Gln Pro Lys Thr Gly Pro Phe Glu Asp Leu Lys Glu
225                 230                 235                 240

Asn Leu Ile Arg Glu Tyr Val Lys Gln Thr Trp Asn Leu Gln Gly Gln
                245                 250                 255

Ala Leu Glu Gln Ala Ile Ile Ser Gln Lys Pro Gln Leu Glu Lys Leu
            260                 265                 270

Ile Ala Thr Thr Ala His Glu Lys Met Pro Trp Phe His Gly Lys Ile
        275                 280                 285

Ser Arg Glu Glu Ser Glu Gln Ile Val Leu Ile Gly Ser Lys Thr Asn
    290                 295                 300

Gly Lys Phe Leu Ile Arg Ala Arg Asp Asn Asn Gly Ser Tyr Ala Leu
305                 310                 315                 320

Cys Leu Leu His Glu Gly Lys Val Leu His Tyr Arg Ile Asp Lys Asp
                325                 330                 335

Lys Thr Gly Lys Leu Ser Ile Pro Glu Gly Lys Lys Phe Asp Thr Leu
            340                 345                 350

Trp Gln Leu Val Glu His Tyr Ser Tyr Lys Ala Asp Gly Leu Leu Arg
        355                 360                 365

Val Leu Thr Val Pro Cys Gln Lys Ile Gly Thr Gln Gly Asn Val Asn
    370                 375                 380
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 224 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg Thr Phe
1               5                   10                  15

Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met Leu Glu
            20                  25                  30

Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg Asn Lys Pro Phe Lys
        35                  40                  45
```

```
Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu Gly Val
    50                  55                  60

Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser Pro Asp
65                  70                  75                  80

Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro His Ala
                85                  90                  95

Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu Gly Leu Val Pro
            100                 105                 110

Arg Gly Ser Met Ala Asn Ser Leu Glu Pro Glu Pro Trp Phe Phe Lys
        115                 120                 125

Asn Leu Ser Arg Lys Asp Ala Glu Arg Gln Leu Leu Ala Pro Gly Asn
    130                 135                 140

Thr His Gly Ser Phe Leu Ile Arg Glu Ser Glu Ser Thr Ala Gly Ser
145                 150                 155                 160

Phe Ser Leu Ser Val Arg Asp Phe Asp Gln Asn Gln Gly Glu Val Val
                165                 170                 175

Lys His Tyr Lys Ile Arg Asn Leu Asp Asn Gly Gly Phe Tyr Ile Ser
            180                 185                 190

Pro Arg Ile Thr Phe Pro Gly Leu His Glu Leu Val Arg His Tyr Thr
        195                 200                 205

Asn Ala Ser Asp Gly Leu Cys Thr Arg Leu Ser Arg Pro Cys Gln Thr
    210                 215                 220
```

WHAT IS CLAIMED IS:

1. A method of screening for compounds capable of binding to a fusion protein which comprises the steps of:

a) mixing a test compound, a tagged ligand, the fusion protein, and a radiolabeled ligand;
b) adding the mixture to a coated microscintillation plate;
c) incubating the mixture for between about 1 hour and about 24 hours;
d) measuring the plate-bound counts attributable to the binding of the tagged ligand to the fusion protein in the presence of the test compound using scintillation counting; and
e) determining the binding of the tagged ligand to the fusion protein in the presence of the test compound relative to a control assay run in the absence of the test compound.

2. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 1, wherein the tagged ligand is a biotinylated ligand or epitope-tagged ligand.

3. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 2, wherein the coated microscintillation plates are streptavidin-coated or anti-antibody or protein A-coated.

4. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 3, wherein the radiolabeled ligand consists of a [^{3}H]-, [^{125}I]-, [^{14}C]-, [^{35}S]-, [^{32}P]-, or [^{33}P]-labeled FK506 analog.

5. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 4, wherein the fusion protein comprises an FK506-binding protein linked through a peptide linker to a target protein.

6. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 5, wherein the target protein comprises a single or multiple signal transduction domain.

7. The method for screening for compounds capable of binding to a fusion protein, as recited in Claim 6, wherein the single or multiple signal transduction domain is selected from the group consisting of: SH1, SH2, SH3 and PH domains.

8. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 7, wherein the target protein is a single or multiple SH2 domain.

9. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 8, wherein the radiolabeled ligand is [^{3}H]-dihydroFK506.

10. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 9, wherein the FK506-binding protein is a 12kDA human FK506-binding protein.

11. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 10, wherein the target protein is a single or multiple SH2 domain selected from the group consisting of: ZAP:SH2, SYK:SH2 and LCK:SH2.

12. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 11, wherein the target protein is the SH2 domain, ZAP:SH2.

13. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 11, wherein the target protein is the SH2 domain, SYK:SH2.

14. The method of screening for compounds capable of binding to a fusion protein, as recited in Claim 11, wherein the target protein is the SH2 domain, LCK:SH2.

15. A process for preparing a recombinant DNA expression vector encoding for a fusion protein comprising the steps of:

a) removing the stop codon on DNA encoding for an FK506-binding protein;

b) synthesizing a modified DNA fragment on the DNA encoding for the FK506-binding protein which encodes for a peptide linker;

c) digesting an expression vector at cloning sites;

d) cloning the modified DNA fragment encoding for the FK506-binding protein with a peptide linker into the digested expression vector to generate a recombinant DNA expression vector encoding for FK506-binding protein with a peptide linker; and

e) cloning DNA encoding for a target protein into a recombinant DNA expression vector encoding for FK506-binding protein with a peptide linker to produce the recombinant DNA expression vector encoding for the fusion protein.

16. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 15, wherein the target protein is a single or multiple signal transduction domain.

17. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 16, wherein the single or multiple signal transduction domain is selected from the group consisting of: SH1, SH2, SH3 and PH domains.

18. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 17, wherein the single or multiple signal transduction domain is an SH2 domain.

19. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 18, wherein the single or multiple signal transduction domain is an SH2 domain selected from the group consisting of ZAP:SH2, SYK:SH2 and LCK:SH2.

20. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 19, wherein the FK506-binding protein is a 12 kDa FK506 binding protein.

21. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 20, wherein the peptide linker has the amino acid sequence GLVPRGS.

22. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 21, wherein the expression vector is selected from the group consisting of: pBR322, pGEX, pT7, pET, pIBI, pProEX-1, pBluescript II, pTZ18R and pTZ19R, pSE420, pVL1392, pBlueBac, pBAcPAK, pHIL, pYES2, pCDNA, and pREP.

23. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 22, wherein the expression vector is the T7 RNA polymerase based pET expression vector.

24. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 23, wherein the target protein is ZAP:SH2.

25. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 23, wherein the target protein is SYK:SH2.

26. The process for preparing a recombinant DNA expression vector encoding the fusion protein, as recited in Claim 23, wherein the target protein is LCK:SH2.

27. Isolated DNA encoding for a fusion protein comprising the sequence:
(SEQ. ID. NO. 1).

28. Isolated DNA encoding for a fusion protein comprising the sequence:
(SEQ. ID. NO. 2).

29. Isolated DNA encoding for a fusion protein comprising the sequence:
(SEQ. ID. NO. 3).

30. A FKBP-ZAP:SH2 fusion protein comprising the sequence:
(SEQ. ID. NO. 4).

31. A FKBP-SYK:SH2 fusion protein comprising the sequence:
(SEQ. ID. NO. 5).

32. A FKBP-LCK:SH2 fusion protein comprising the sequence:
(SEQ. ID. NO. 6).

33. A process for expressing recombinant DNA encoding for a fusion protein in an expression vector comprising the steps of:

a) transforming a host cell with the fusion protein expression vector;
b) inducing expression of the fusion protein in the host cell;
c) recovering the fusion protein from the host cell; and
d) purifying the fusion protein.

34. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 33, wherein the target protein is a single or multiple signal transduction domain.

35. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 34, wherein the single or multiple signal transduction domain is selected from the group consisting of: SH1, SH2, SH3 and PH domains.

36. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 35, wherein the single or multiple signal transduction domain is a single or multiple SH2 domain.

37. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 36, wherein the single or multiple SH2 domain is selected from a group consisting of ZAP:SH2, SYK:SH2 and LCK:SH2.

38. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 37, wherein the FK506-binding protein is human 12kDa FK506-binding protein.

39. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 38, wherein the host cell is from bacteria, yeast, blue green algae, plant cells, insect cells, or animal cells.

40. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 39, wherein the expression vector is T7 RNA polymerase based expression vector.

41. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 40, wherein the host cell is an *E. coli* strain selected from a group consisting of BL21 (DE3), Nova Blue (DE3), and JM109 (DE3).

42. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 41, wherein the single or multiple SH2 domain is ZAP:SH2.

43. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 41, wherein the single or multiple SH2 domain is SYK:SH2.

44. The process for expressing recombinant DNA encoding a fusion protein, as recited in Claim 41, wherein the single or multiple SH2 domain is LCK:SH2.

45. The process for purifying an isolated FKBP-SH2 fusion protein comprising the steps of:

a) preparing an affinity matrix consisting of biotinylated phosphopeptide coupled to avidin or streptavidin immobilized on a solid support;
b) preparing a freeze/thaw extract from cells expressing the fusion protein;
c) loading the extract onto the affinity matrix and washing off unbound protein; and
d) eluting the desired fusion protein with phenyl phosphate.

46. A recombinant FKBP-SH2 domain T7 RNA polymerase-based expression vector, wherein the DNA encodes for the FKBP-ZAP:SH2 fusion protein and has the DNA sequence (SEQ. ID. NO. 1).

47. A recombinant FKBP-SH2 domain T7 RNA polymerase-based expression vector, wherein the DNA encodes for the FKBP-SYK:SH2 fusion protein and has the DNA sequence (SEQ. ID. NO. 2).

48. A recombinant FKBP-SH2 domain T7 RNA polymerase-based expression vector, wherein the DNA encodes for the FKBP-LCK:SH2 fusion protein and has the DNA sequence (SEQ. ID. NO. 3).

49. A recombinant host cell containing the recombinant FKBP-SH2 domain T7 RNA polymerase-based expression vector wherein the recombinant host cell isselected from the group consisting of: *E. coli* BL21 (DE3), *E. coli* Nova Blue (DE3), and *E. coli* JM109 (DE3).

50. The recombinant host cell containing the recombinant FKBP-SH2 domain T7 RNA polymerase-based expression vector as recited in claim 49, wherein the recombinant host cell is *E. coli* BL21 (DE3).

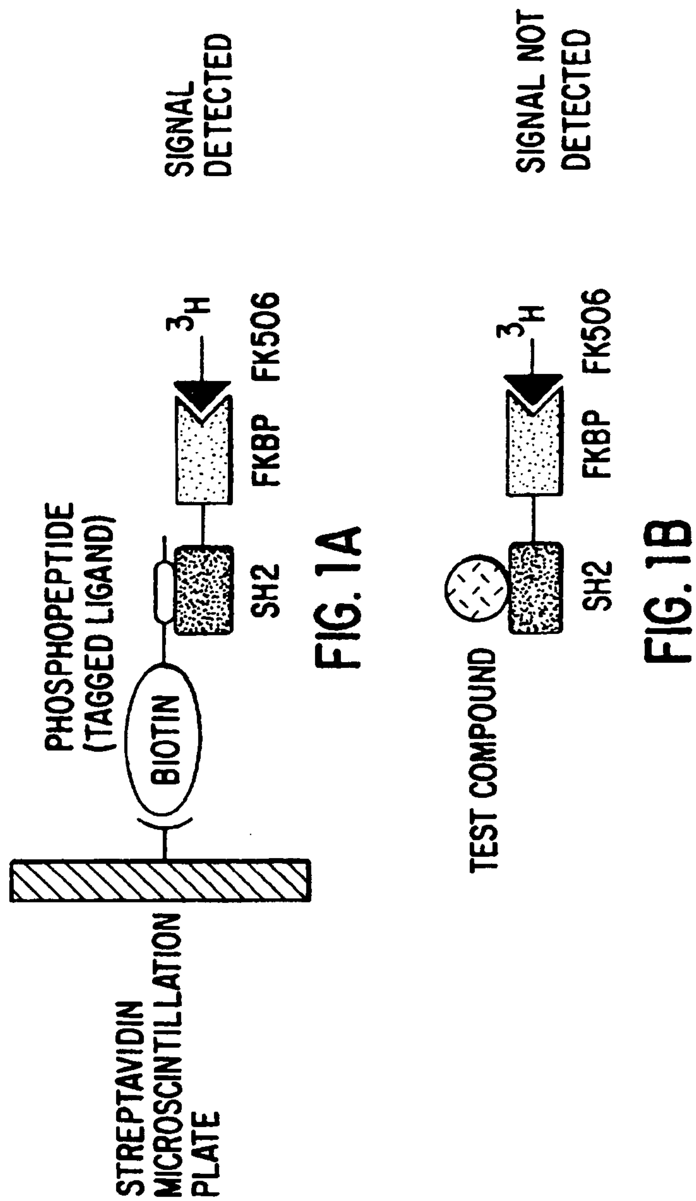
STREPTAVIDIN MICROSCINTILLATION PLATE
BIOTIN
PHOSPHOPEPTIDE (TAGGED LIGAND)
SH2
FKBP
FK506
3H
SIGNAL DETECTED
TEST COMPOUND
SH2
FKBP
FK506
3H
SIGNAL NOT DETECTED

FIG. 1A

FIG. 1B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14567

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.7, 70.1, 70.3, 71.1, 71.2, 172.3, 252.33, 320.1; 436/501; 530/350, 413; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P --- Y,P	US 5,498,597 A (BURAKOFF et al.) 12 March 1996, column 12, lines 28-67.	15 --------- 1-14, 16-50
Y,P	US 5,464,745 A (MIERENDORF et al.) 07 November 1995, column 7, lines 36-42.	1-14
A	US 5,434,064 A (SCHLESSINGER et al.) 18 July 1995.	1-50
Y,E	US 5,580,979 A (BACHOVCHIN) 03 December 1996, column 4, lines 10-19.	11-14, 24-32, 37-44, 46-50
X -- Y	US 5,352,660 A (PAWSON) 04 October 1994, column 8, lines 29-58; column 9, lines 15-26; column 10, lines 39-42; column 11, lines 11-20.	33-36 ------ 1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 DECEMBER 1996	31 JAN 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	BRIAN K. LATHROP JAB for
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14567

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,534,424 A (UHLEN et al.) 09 July 1996, Fig. 1.	2-14
Y	STUDIER et al. Use of T7 RNA polymerase to direct expression of cloned genes. Methods in Enzymology. 1990, Vol. 185, pages 60-89, the entire document.	22-32, 39-44, 46-50
A,P	SONATORE et al. The utility of FK506-binding protein as a fusion partner in scintillation proximity assays: application to SH2 domains. Analytical Biochemistry. 05 September 1996, Vol. 240, No. 2, pages 289-297.	
X -- Y	GILMER et al. Peptide inhibitors of src SH3-SH2- phosphoprotein interactions. The Journal of Biological Chemistry. 16 December 1994, Vol. 269, No. 50, pages 31711-31719, the entire document.	33-36 -------- 1-32, 37-50
A,P	MULLER et al. Rapid identification of phosphopeptide ligands for SH2 domains: screening of peptide libraries by fluorescence-activated bead sorting. The Journal of Biological Chemistry. 12 July 1996, Vol. 271, No. 28, pages 16500-16505.	1-50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14567

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 21/04; C07K 14/00, 14/195, 14/435, 17/00, 17/06, 17/14; C12N 1/21, 15/00, 15/09, 15/63, 15/70; C12P 21/00, 21/02; G01N 33/53, 33/547, 33/566; A23J 1/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.7, 70.1, 70.3, 71.1, 71.2, 172.3, 252.33, 320.1; 436/501; 530/350, 413; 536/23.4

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

USPAT, WPIDS, INPADOC, MEDLINE, SCISEARCH, CAPLUS, EMBASE
search terms: FKBP, SH2, fusion, LCK, ZAP, SYK, streptavidin, biotin, screen###, coat###, scintillation, Marcy, A., Salowe, S., Wisniewski, D.